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(71)(72) Applicant and Inventor: BOMAN, Hans, G. [SE/SE]; Odengatan 23, S-114 24 Stockholm (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: AGERBERTH, Birgitta [SE/SE]; Ångsklockevägen 43, S-181 57 Lidingö (SE). GUDMUNDSSON, Gudmundur, H. [SE/SE]; Kungshamra 43A, S-170 70 Solna (SE). GUNNE, Hans [SE/SE]; Drottninggatan 90, S-111 36 Stockholm (SE).

(74) Agent: AWAPATENT AB; P.O. Box 45086, S-104 30 Stockholm (SE).

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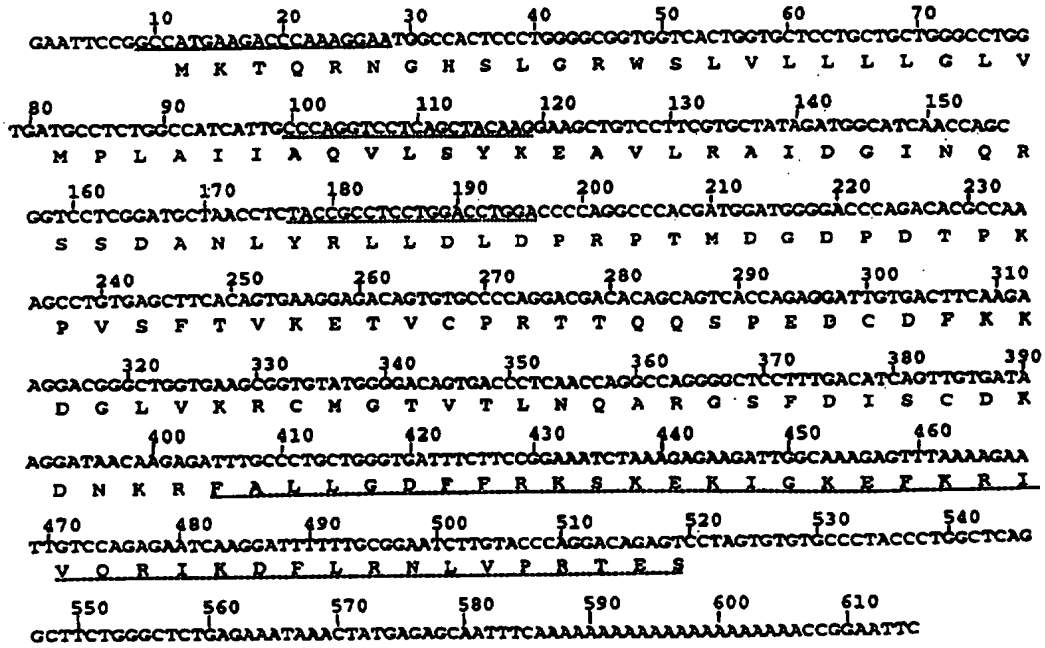

(57) Abstract

New polypeptides comprising the following amino acid sequence: E K I G K E F K R I V Q R I K D F L R N L V, and functional derivatives and pharmaceutically acceptable salts thereof; pharmaceutical composition containing such polypeptides as active ingredients; a method for inhibiting microbial growth in animals including man; and a cDNA sequence capable of expressing a precursor protein to such polypeptide.

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A new human peptide antibiotic (FALL-39) and its use

The present invention relates to a new human polypeptide (FALL-39), its therapeutic use, pharmaceutical compositions containing same as an active ingredient, a cDNA sequence, and a method for inhibiting microbial growth.

Animal peptide antibiotics were discovered about 15 years ago and in September 1993 at least some 50 different sequences were known (for reviews see Goode, J. ed (1994) *Antimicrobial Peptides, Ciba Symposium No. 186.* (John Wiley & Sons Ltd. Chichester, PO19 1UD, UK); Boman, H.G. (1995) Annu. Rev. Immunol. 13, in press). On a chemical basis, these peptides can be divided into five groups:

i) Linear peptides without Cys, often forming amphipathic helices,

ii) Linear peptides with a high proportion of certain residues like Pro and Arg,

iii) Loop-forming peptides with one disulfide bond,

iv) Peptides with two or more disulfide bonds, normally forming β-sheet structures, and

v) Peptides derived from larger molecules with other known functions.

The peptides most studied are the cecropins and the magainins in the first group and the defensin family in the fourth group.

On a functional basis, the animal peptide antibiotics can be divided into two groups: Those which like the defensins accumulate in the granule of phagocytes and those which are delivered into body fluids or epithelial layers. Peptides which have evolved to kill engulfed microbes inside phagocytic vacuoles can in released form be cytotoxic to the host and this is the case for the defensins (Lehrer, R.I., Lichtenstein, A.K. and Ganz, T. (1993) *Annu. Rev. Immunol.* 11, 105-128). On the other hand peptides like the insect cecropins (Boman, H.G., Faye, I., Gudmundsson, G.H., Lee, J.-Y. and Lidholm, D.A. (1991)

Eur. J. Biochem. 201, 23-31) and insect defensins (Hoffmann, J.A. and Hetru, C. (1992) *Immunol. Today 13*, 411-415) which are delivered into the circulatory system are not harmful to the producing organism.

Animal peptide antiobiotics differ from the "classical" antibiotics in several respects (Boman, H.G. (1995) *Annu. Rev. Immunol. 13*, in press; Boman, H.G. (1994) in *Antimicrobial Peptides, Ciba Symposium No. 186*, pp 1-4, ed. Goode, J. (John Wiley & Sons Ltd. Chichester, PO19 1UD, UK)). The animal peptides are all gene encoded and they are made as preproproteins which are processed to the mature peptide by defined pathways. The actual processing steps have been studied for cecropins (Boman, H.G., Boman, I.A., Andreu, D., Li, Z.-q., Merrifield, R.B., Schlenstedt, G. and Zimmermann, R. (1989) *J.Biol. Chem. 264*, 5852-5860), for Bac5 and Bac7 (Scocchi, M., Skerlavaj, B., Romeo, D. and Gennaro, D. (1992) *Eur.J.Biochem. 209*, 589-595) and for myeloid defensins (Ganz, T., Liu, L., Valore, E.V. and Oren, A. (1993) *Blood 82*, 641-650), but in most other cases the processing is so far only predicted or simply unknown. This biosynthesis is conceptually different from the one for microbial peptide antibiotics like gramicidin or penicillin which are made by a set of different enzymes that sequentially add different amino acid residues. Animal and microbial antibiotics differ also functionally: Microbial anticiotics are often referred to as "secondary metabolites" (Chadwick, D.J. and J. Whelan, ed. (1992) *Secondary Metabolites: Their Function and Evolution. Ciba Foundation Symposium 171.* (John Wiley & Sons Ltd., Chichester, PO19 1UD, UK) while the animal peptide antibiotics are considered as important parts of the innate immunity of the producing organism (Boman, H.G. (1995) *Annu. Rev. Immunol. 13*, in press).

So far, most of the animal peptide antibiotics have been purified from blood (hemolymph) or blood cells using as assay the antimicrobial activity. cDNA and genomic clones were isolated later with the help of probes desig-

ned from the known amino acid sequences. However, Romeo and Zanetti discovered that a number of antibacterial peptides from different mammals contained a conserved proregion very similar to cathelin (Zanetti, M., Del Sal, G., Storici, P., Schneider, C. and Romeo, D. (1993) *J. Biol.Chem. 266*, 522-526), a protein isolated from pig leucocytes and reported to be an inhibitor of Cys-containing proteases (Ritonja, A., Kopitar, M., Jerala, R. and Turk, V. (1989) *FEBS Lett. 255*, 211-214). This finding was used by the Trieste groups for 3'- and 5'-RACE experiments that gave the cDNA sequences corresponding to both previously known peptide antibiotics (Storici, P. and Zanetti, M (1993) *Biochem. Biophys. Res. Commun. 196*, 1363-1368; Del Sal, G., Storici, P., Schneider, C., Romeo,D. and Zanetti, M. (1992) *Biochem. Biophys. Res. Com. 187*, 467-472; Storici, P. and Zanetti, M (1993) *Biochem. Biophys. Res. Comm. 196*, 1058-1065) and to novel molecules (PMAP-36) which were synthesized and found to be antibacterial (Storici, P., Scocchi, M., Tossi, A., Gennaro, R. and Zanetti, M. (1994) *FEBS Lett 337*, 303-307).

It is generally known that resistance to classical antibiotics is becoming an increasing clinical problem. The present invention has for its main object to provide new polypeptides of biological activity but different from classical antibiotics, such as penicillins and tetracyclins.

Another object of the invention is to provide new human polypeptides having antimicrobial activity against bacteria without being cytotoxic.

Yet another object of the invention is to provide pharmaceutical compositions containig such polypeptides as active ingredients.

Still another object of the invention is to provide a method for inhibiting microbial growth in animals including man.

A further object of the invention is to provide the clone enabling expression of a precursor protein for the new polypeptides.

For these and other objects which will be clear from the following disclosure the invention provides for a polypeptid, comprising the following amino acid sequence:

E K I G K E F K R I V O R I K D F L R N L V

including also functional derivatives and pharmaceutically acceptable salts thereof.

Another aspect of the invention is reflected by the provision of a polypeptide comprising the following amino sequence:

F A L L G D F F R K S K E K I G K E F K R I V O R I K D F L R N L V P R T E S

also including functional derivatives and pharmaceutically acceptable salts thereof.

The new polypeptides according to the invention find therapeutic applications, such as used as antimicrobial agents, such as antibacterial agents.

The invention also includes pharmaceutical compositions containing as active ingredients one or more of the polypeptides according to the invention in an effective amount together with a pharmaceuticallly acceptable carrier or diluent.

Said amount is suitably antimicrobially active, such as antibacterially active. The carrier or diluent is preferably adapted for oral, intravenuous, intramuscular or subcutaneous administration. Finally, the invention includes a method for inhibiting microbial growth in animals, such as mammals including man, said method comprising the step of administering to an animal subject to a disorder caused by microbial attack a polypeptide as defined above, a functional derivative or a pharmaceutically

acceptable salt thereof, or a composition as defined above in an inhibitory amount.

Such method can be directed to intestinal use constituted by oral administration of a composition as defined above in a slow release form. The method can also be directed to administration by injection of such a composition in an injectable dose form.

With regard to the expression "functional derivatives thereof" it is well known in regard to the technical area to which the present invention pertains that minor amino acid substitutions can be made to the polypeptide which do not affect or do not substantially affect the function of the polypeptide. Determination of conceivable substitutions is accomplished according to procedures well known to those skilled in the art. Thus, all polypeptides having substantially the same amino acid sequence, substantially the same helical structure and substantially the same biological activity, such as antimicrobial and lytic activity, are within the scope of this invention.

Also within the scope of the present invention are pharmaceutically acceptable salts of the polypeptides of this invention. Such salts are formed by methods well known to skilled artisans. Thus, for example base salts of the polypeptides can be prepared according to conventional methods. When in the instant disclosure including the claims the term polypeptide is used said term is intended to include both functional derivatives and pharmaceutically acceptable salts of the polypeptides.

The active polypeptide according to the present invention can be formulated for use in human or veterinary medicine for therapeutic or prophylactic use. The active preparations are normally administered orally, rectally or parenterally, such as by injection in the form of a pharmaceutical preparation or composition comprising the active constituents in combination with a pharmaceutically acceptable carrier which may be solid, semi-solid or liquid, or contained in a capsule, such as when orally admi-

nistered. The administration may also take the form of topical application. As examples of pharmaceutical preparations there may be mentioned tablets, drops, solutions and suppositories. Usually, the active constituent constitutes the minor part of the preparation, such as from about 0.1 to about 50% thereof based on weight.

In order to prepare pharmaceutical compositions in the form of dose units for oral application the polypeptide of the invention can be mixed with a solid, pulverulent or other carrier, for example lactose, saccharose, sorbitol, mannitol, starch, such as potatoe starch, corn starch, millopectine, cellulose derivative or gelatine, and may also include lubricants, such as magnesium or calcium stearate, or polyethylene glycol waxes compressed to the formation of tablets or bodies for dragées. The dose units may also be presented in a coated form of enteric type.

By using several layers of the carrier or diluent tablets operating with slow release can be prepared.

Liquid preparations for oral application or for injection can be made in the form of elexirs, syrups or suspensions, for example solutions containing from 0.1 to 20% by weight of active substance, sugar and a mixture of ethanol, water, glycerol, propyleneglycol and possibly other additives of a conventional nature.

The scientific research from which the present invention has emerged, have shown that the gene for the peptide FALL-39 is expressed mainly in bone marrow and in testis, both of which organs are not often infected. An important aspect of the present invention is the use of the polypeptides of the invention in products for the treatment of urinary tract infections and sexually transmitted diseases, such as disorders created by clamydia, gonococchi and HIV virus. Such treatment can be performed by using a solution of the antimicrobially active polypeptide in lavage of invaded organs, cavities or tubes, such as the urinary tract, the bladder, urethral organs and passages, renal

organs and associated passages, male genital organs and associated passages, etc.

The dose by which the active constituent is administered may vary within wide limits and is dependent on different factors, such as the seriousness of the disorder, the age and the weight of the patient and can be adjusted individually.

The present invention will now be described by non-limiting examples through the following disclosure. In this disclosure the new 39 residue polypeptide will be designated FALL-39, the peptide being named after the first four amino acid residues of the peptide. The following disclosure will be made with reference to the appended drawings, wherein:

Figure 1 shows the cDNA sequence for prepro-FALL-39 with translation of the open reading frame;

Figure 2 illustrates two Northern blot analyses with RNA from a leucemic child, a healthy child and a child with leucemia in remission in comparison with mRNA from human bone marrow;

Figure 3 shows an Edmundson wheel plot for residues E13-V34 in the sequence of FALL-39;

Figure 4 shows CD spectra of a water solution of synthetic FALL-39 before and after adding of medium E; and

Figure 5 shows an inhibition zone assay for FALL-39 on E.coli.

Materials and Methods

cDNA Cloning

A liquid lysate of a human bone marrow λgt11 cDNA library (Clontech) was used to isolate template DNA by Wizard DNA purification system (Promega). The following three primers, 5'ACCATGGAGACCCAGAGGGC, 5'CCTGTAGCTGAGGGCCTGGG and 5'TCCA(A/G)(C/T)TCCA(A/G)CA(A/G)(A/G/C/T)C(G/T)(A/G)TA, (corresponding to underlined or dashed sequences in Fig. 1) were directed to the signal sequence and the proregion

of PR-39. These primers (at 0.4 µM) and template DNA (6ng/µl) were used in a PCR experiment with the following thermal cycle profile; 95°C 3 min, 40 cycles of 95°C 1 min, 55°C 1 min, 72°C 1 min and an extension step of 72°C 7 min. Analyses of the reaction mixtures showed two bands of the expected sizes. The bands were purified and cloned into the pCR™II vector by a TA cloning kit (Invitrogen). Positive clones were sequenced by the dideoxy method with a sequenase kit (U.S. Biochemicals). Sequencing confirmed that the two PCR bands were similar to the start of the open reading frame for preproPR-39. The larger band (183 bp) was radioactively labelled and used as a probe for screening of a human bone marrow cDNA library (λgt11 from Clontech). About 150 000 pfu were screened using Hybond-N nylon membrane (Amersham) and positive plaques were purified to homogeneity. λDNA was prepared by a glycerol step gradient (Maniatis, T., Fritsch, E.F. and Sambrook, J. (1989) *Molecular cloning. A laboratory manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor)). The cDNA inserts were subcloned into pBluescript KS vector (Stratagene) and sequenced by the solid-phase sequencing method (Hultman, T., Bergh, S. and Uhlén, M. (1991) *Bio Techniques 10*, 84-93) on an A.L.F. (Pharmacia, Sweden) and an Applied Biosystems sequenator. The screening hybridization was done in 6xSSC, 5xDenhardt's, 1% SDS and 100 µg/ml of denatured herring sperm DNA at 55°C overnight. Final washing was in 2xSSC and 0.1% SDS at 55°C.

Nucleic acid analysis

Total RNA was isolated by an RNA separator kit (Clontech) and two filters preloaded with mRNA from different human tissues (Clontech) were used for hybridization. RNA was separated by electrophoresis in a denaturating formaldehyde gel and the hybridizations were carried out under high stringency conditions (Maniatis T., Fritsch, E.F. and Sambrook, J. (1989) *Molecular cloning. A laboratory manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

Peptide synthesis

Chemical peptide synthesis was carried out with an automatic peptide synthesizer (Applied Biosystem 430A) using standard solid-phase procedure (reviewed by Merrifield (Merrifield, R.B. (1986) *Science* 232, 341-347). Starting from t-BOC-Ser(benzyl)-OCH_2-PAM resin (0.67 mmol/g), t-BOC amino acid derivatives were used with reactive side chains protected as follows: Ser and Thr with benzyl, Lys with 2-chlorobenzyl-oxycarbonyl, Glu and Asp with benzyl ester, Arg with 4-toluene-sulfonyl. A standard program with preformed symmetric anhydrides and preformed 1-hydroxybenzotriazole-esters was used for the synthesiss. Double couplings were carried out for Arg, Gln and Asn. The completed 39-residue peptide was cleaved from the resin with liquid hydrogen fluoride:anisole:methyl sulfide (10:1:1) for 60 min at 0°C. The cleavage product was washed with ether in order to remove the scavengers and then extracted into 30% acetic acid and lyophilized. The peptide was purified by HPLC on a Vydac C18 column, utilizing a linear gradient of 80% acetonitrile (20-75% for 30 min) in 0.1% trifluoroacetic acid. The molecular mass was analysed by a time-of-flight mass spectrometer (Biolon 20). The CD spectra were recorded with a J-710 spectropolarimeter (JASCO, Japan) at the BioScience Center of Pharmacia.

Antibacterial assay.

The antibacterial activity was recorded with the inhibition zone assay and the final result obtained from a dilution series is given as the lethal concentration (the LC-value, the lowest concentration that would inhibit bacterial growth (Hultmark, D., Engström, A., Andersson, K., Steiner, H., Bennich, H. and Boman, H.G. (1983) *EMBO J.* 2, 571-576)).

EXAMPLE 1

Isolation of a cDNA clone for FALL-39 and its characteristics.

Initially 9 primers were designed using both sequence information from the porcine gene for prepro-PR-39 (Agerberth, B., Lee, J-Y., Bergman, T., Carlquist, M., Boman, H.G., Mutt, V., Jörnvall, H. (1991) *Eur.J.Biochem. 202*, 849-854) and conserved regions of the preprosequences in all published sequences in the "cathelin family" (Storici, P. and Zanetti, M. (1993) *Biochem. Biophys. Res. Commun. 196*, 1363-1368; Del Sal, G., Storici, P., Schneider, C., Romeo, D. and Zanetti, M. (1992) *Biochem. Biophys. Res. Com. 187*, 467-472; Storici, P. and Zanetti, M. (1993) *Biochem. Biophys. Res. Comm. 196*, 1058-1065); Storici, P., Scocchi, M., Tossi, A., Gennaro, R. and Zanetti, M. (1994) *FEBS Lett. 337*, 303-307). A total of 9 primers in 11 combinations were utilized for PCR, using as template DNA froma human bone marrow cDNA library. Analyses of these 11 reaction mixtures showed that only two combinations gave clear bands of the expected sizes. Cloning and sequencing showed both bands to be cathelin-like in structure. As the next step the larger band (183 bp) was used as a probe to screen a human bone marrow library. A number of positive clones were isolated. Partial sequences of the inserts indicated that all clones contained information for the preproform of a novel 39-residue peptide, FALL-39.

EXAMPEL 2

Eight positive clones were fully sequenced and gave the same cDNA structure for FALL-39 as shown in Fig. 1 illustrating cDNA sequence for prepro-FALL-39 with translation of the open reading frame. The putative peptide FALL-39 is indicated by a dashed underlining. The regions towards which the three succesful primers were directed are indicated as a full line for the corresponding sequence and dotted lines for the two complementary sequences. The cathelin sequence starts with bases 101-115, translated to QVLSY. * is a stop signal. A comparison with the genomic DNA sequence for PR-39 shows that both the signal

sequences and the propart are partly conserved. However, the C-terminal ends of the cathelin parts differ in the six last residues: in prepro-FALL-39 the end is a typical dibasic cleavage site (KR, residue 130-131) instead of an elastase site (SV) in prepro-PR-39. The mature peptides are also totally different, there is no homology at all and the motifs PRP and PP, typical of proline-arginine-rich peptides, are absent in FALL-39. A search in GenBank for sequences with similarity to FALL-39 gave no significant relations to published peptides or proteins. It is therefore concluded that the putative peptide FALL-39 has a novel sequence.

EXAMPLE 3

Three Northern blot analyses were carried out. One with a commercial sample of mRNA from human bone marrow and three samples of total RNA prepared from different human bone marrow samples. In addition we have used two commercial filters preloaded with mRNA from 16 different human tissues. The two filters that gave signals are shown in Fig. 2 showing two Northern blot analyses: (a) with total RNA from bone marrow from a child with T cell leucemia (A), a healthy child (B) and child with leucemia in remission (C) compared to mRNA from human bone marrow (Clontech); (b) a commercial preloaded filter with human mRNA from spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leucocytes (PBL). For both blots an actin probe was used to demonstrate the amounts of RNA applied. Clear signals were obtained only for bone marrow and testis from healthy individuals. Overexposure of the film in Fig. 2 (a) showed a faint signal from peripheral blood leukocyte RNA. A filter preloaded with human mRNA from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas gave no signals.

EXAMPLE 4

Solid-phase synthesis of FALL-39 and a study of its properties.

FALL-39 was synthesized with residues 132-170 of the precursor structure. The synthetic peptide was analysed in a time-of-flight mass spectrometer and found to have an M_r=4707.9. The peak was slightly asymmetric indicating some decomposition during the run. The mass value is therefore in reasonable agreement with the calculated value of 4711.6 for FALL-39.

An Edmundson wheel plot of the FALL-39 sequence showed that the central part of the molecule (residues 13-34) could form a perfect amphipathic helix as seen in Fig. 3, a property often found for antibacterial peptides like the cecropins and the magainins. Fig. 3 shows an Edmundson wheel plot for residues E13-V34 in the sequence for FALL-39. The dotted line divides the helix into a hydrophilic and a hydrophobic part. The start of the helix is at the top of the wheel. A plot of residues F7-V34 does not give a perfect amphipathic helix. The synthetic peptide was investigated for its helix content by CD spectroscopy. The spectra of an 86 µM solution of synthetic FALL-39 in water indicated a lack of structure (random coil) while the addition of Medium E induced about 30% of helix formation in the peptide as seen in Fig. 4. Fig. 4 shows CD spectra of a water solution of synthetic FALL-39 (86 µM) and the same solution after adding 2% of 50 times concentrated Medium E. Values below 190 nm are believed to be artifacts from the solvents. About 50% of helix formation was induced in the presence of 30% trifluoroethanol.

EXAMPLE 5

Preliminary experiments showed FALL-39 to be active against Escherichia coli and Bacillus megaterium, providing that the basal Medium E (MedE) (Vogel, H.J. and D.M. Bonner (1956) *J.Biol.Chem. 218*, 97-106) was added to the LB plates (which contains 0.9% NaCl). Fig. 5 shows the inhibition zones produced by FALL-39 on E.coli D21 accor-

ding to Hultmark (Hultmark, D., Engström, A., Andersson, K., Steiner, H., Bennich, H. and Boman, H.G. (1983) *EMBO J. 2*, 571-576). The bacteria were grown in thin agarose plates with LB medium supplemented with basal medium E (MedE). The zone area should normally be a function of the log of the amounts of peptide applied to each well as shown in the upper curve. In the absence of MedE there is a concentration dependance only for the three highest amounts of peptide. The graph also shows the zones obtained with our standard assay conditions. The LC-value (Hultmark, D., Engström, A., Andersson, K., Steiner, H., Bennich, H. and Boman, H.G. (1983) *EMBO J. 2*, 571-576) obtained from the data in the upper curve was 0.7 µM while a calculation based on the three highest amounts of peptide in LB medium gave an LC-value of 8.9 µM. A similar salt dependance was found also for the action of FALL-39 on B. megaterium Bm11 (data not shown) giving LC-values of 0.2 µM in MedE. The LC-values obtained for the porcine peptides cecropin P1 and PR-39 on E.coli D21 were 0.4 and 0.3, respectively (Boman, H.G., Agerberth, B. and Boman, A. (1993) *Infect. Immun. 61*, 2978-2984). Flat and non-linear concentration dependances as seen for the standard assay conditions in Fig. 4 are difficult to interpret and the LC-value from the three highest amounts may be misleading. The wells in the bacterial plates with the highest amounts of FALL-39 sometimes showed a white halo at the edges which could indicate that the peptide was bound to the agarose. FALL-39 did not give any detectable lysis of human red cells. The presence of 10 µM of FALL-39 during five days of incubation did not affect the incorporation of ^{3}H-thymine in triplicate cultures of human peripheral blood lymphocytes (PBL) stimulated with PHA. Thus, FALL-39 does not seem to be cytotoxic for human cells.

The present invention relates to the provision of novel human antibacterial peptides which are free of cystein. The sequence of the clone for prepro-FALL-39 indicates that the molecule belongs to the family of cathelin-

like precursors. In the peptide FALL-39 residues 13-34, inclusive, are capable of forming a perfect amphiphatic helix (cf. Fig. 4). It is obvious that the addition of Medium E to a water solution of FALL-39 induces helix formation in accordance with Fig. 4 and at the same time the activity against E.coli is dramatically improved (cf. Fig. 5). It is therefore concluded that the helix formation is of importance for the biological activity of FALL-39. Medium E is a basal salt medium designed specially for E.coli (Vogel, H.J. and D.M. Bonner (1956) *J.Bio.Chem. 218*, 97-106).

As previously indicated resistance to classical antibiotics is becoming an increasing clinical problem (cf. Chin, G.J. and Marx, J. ed. (1994) *Science 264*, 359-393). This is particularly so since vancomycin resistance recently has been found in clinical isolates (Swartz, M.N. (1994) *Proc. Natl. Acad. Sci. USA 91*, 2420-2427). Against this background the new human peptides according to the present invention are of special interest. The LC-value for FALL-39 on E.coli D21 is 0.7 μM and is comparable to tetracycline having an LC-value in the same assay of 0.9 μM (Boman, H.G., Agerberth, B. and Boman, A. (1993) *Infect.Immun. 61*, 2978-2984). No cytotoxic properties have been found for the peptides according to the present invention. The fact that the peptides of the invention are cystein free makes synthesis easier and cheaper. The peptides thus constitute an important progress in the field of antibacterially active agents for human use.

PATENTKRAV

1. A polypeptide comprising the following amino acid sequence:

E K I G K E F K R I V O R I K D F L R N L V

and functional derivatives and pharmaceutically acceptable salts thereof.

2. A polypeptide (FALL-39) comprising the following amino acid sequence:

F A L L G D F F R K S K E K I G K E F K R I V O R I K D F L R N L V P R T E S

and functional derivatives and pharmaceutically acceptable salts thereof.

3. A polypeptide according to claim 1 or 2 for therapeutic use.

4. A polypeptide according to claim 3 for use as an antimicrobial agent.

5. A polypeptide according to claim 4 for antibacterial use.

6. A pharmaceutical composition containing as an active ingredient the polypeptide according to claim 1 or 2 in an effective amount together with a pharmaceutically acceptable carrier or diluent.

7. A pharmaceutical composition according to claim 4, wherein said amount is antimicrobially active.

8. A pharmaceutical composition according to claim 5, wherein said amount is antibacterially active.

9. A pharmaceutical composition according to any of claims 6 to 8, wherein said carrier or diluent is adapted for oral, intravenous, intramuscular or subcutaneous administration.

10. A method for inhibiting microbial growth in animals, such as mammals including man, comprising the step of administering to an animal subject to a disorder

caused by microbial attack a polypeptide according to claim 1 or 2 or a composition according to any of claims 6 to 9 in an inhibitory amount.

11. A method according to claim 10 for inhibiting bacterial growth.

12. A method according to claim 10 or 11, comprising administration by injection of a composition according to any of claims 6 to 9 in an injectable dose form.

13. A cDNA clone having the following sequence:

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                                                                       60
GAATTCCGGCCATGAAGACCCAAAGGAATGGCCACTCCCTGGGGCGGTGGTCACTGGTGCTCCTGCTGCTGGGCCTGG

                                                                      140
TGATGCCTCTGGCCATCATTGCCCAGGTCCTCAGCTACAAGGAAGCTGTCCTTCGTGCTATAGATGGCATCAACCAGC

                                                                      220
GGTCCTCGGATGCTAACCTCTACCGCCTCCTGGACCTGGACCCCCAGGCCCCACGATGGATGGGGACCCAGACACGCCAA

                                                                      300
AGCCTGTGAGCTTCACAGTGAAGGAGACAGTGTGCCCCAGGACGACCAGCCAGTCACCCAGAGGATTGTGTGACTTCAAGA

                                                                      380
AGGACGGGCTGCTGCTGAAGCCGGTGTATGGGGACAGTGACCCTCAACCAGGCCAGGGCTCCTTTGACATCAGTTGTGATA

                                                                      460
AGGATAACAAGAGATTTGCCTGCTGGGTGATTTCTTCCGGAAATCTAAGAGAAGATTGGCAAAGAGTTTAAAAGAA

                                                                      540
TTGTCCAGAGAATCAAGGATTTTTTGCGGAATCTTGTACCCAGGACAGAGTCCTAGTGTGCCCTAACCCTGGCTCAG

                                                                      610
GCTTCTGGGCTCTGAGAAATAAACTATGAGAGCAATTTCAAAAAAAAAAAAAAAAAAAACCGGAATTC
```

and functional derivatives thereof.

```
          10         20         30         40         50         60         70
GAATTCCGGCCATGAAGACCCAAAGGAATGGCCACTCCCTGGGGCGGTGGTCACTGGTGCTCCTGCTGCTGGGCCTGG
           M  K  T  Q  R  N  G  H  S  L  G  R  W  S  L  V  L  L  L  L  G  L  V

 80         90        100        110        120        130        140        150
TGATGCCTCTGGCCATCATTGCCCAGGTCCTCAGCTACAAGGAAGCTGTCCTTCGTGCTATAGATGGCATCAACCAGC
  M  P  L  A  I  I  A  Q  V  L  S  Y  K  E  A  V  L  R  A  I  D  G  I  N  Q  R

 160        170        180        190        200        210        220        230
GGTCCTCGGATGCTAACCTCTACCGCCTCCTGGACCTGGACCCCAGGCCCACGATGGATGGGGACCCAGACACGCCAA
  S  S  D  A  N  L  Y  R  L  L  D  L  D  P  R  P  T  M  D  G  D  P  D  T  P  K

 240        250        260        270        280        290        300        310
AGCCTGTGAGCTTCACAGTGAAGGAGACAGTGTGCCCCAGGACGACACAGCAGTCACCAGAGGATTGTGACTTCAAGA
  P  V  S  F  T  V  K  E  T  V  C  P  R  T  T  Q  Q  S  P  E  D  C  D  F  K  K

 320        330        340        350        360        370        380        390
AGGACGGGCTGGTGAAGCGGTGTATGGGGACAGTGACCCTCAACCAGGCCAGGGGCTCCTTTGACATCAGTTGTGATA
  D  G  L  V  K  R  C  M  G  T  V  T  L  N  Q  A  R  G  S  F  D  I  S  C  D  K

 400        410        420        430        440        450        460
AGGATAACAAGAGATTTGCCCTGCTGGGTGATTTCTTCCGGAAATCTAAAGAGAAGATTGGCAAAGAGTTTAAAAGAA
  D  N  K  R  F  A  L  L  G  D  F  F  R  K  S  K  E  K  I  G  K  E  F  K  R  I

 470        480        490        500        510        520        530        540
TTGTCCAGAGAATCAAGGATTTTTTGCGGAATCTTGTACCCAGGACAGAGTCCTAGTGTGTGCCCTACCCTGGCTCAG
  V  Q  R  I  K  D  F  L  R  N  L  V  P  R  T  E  S

 550        560        570        580        590        600        610
GCTTCTGGGCTCTGAGAAATAAACTATGAGAGCAATTTCAAAAAAAAAAAAAAAAAAAAAACCGGAATTC
```

Fig. 1

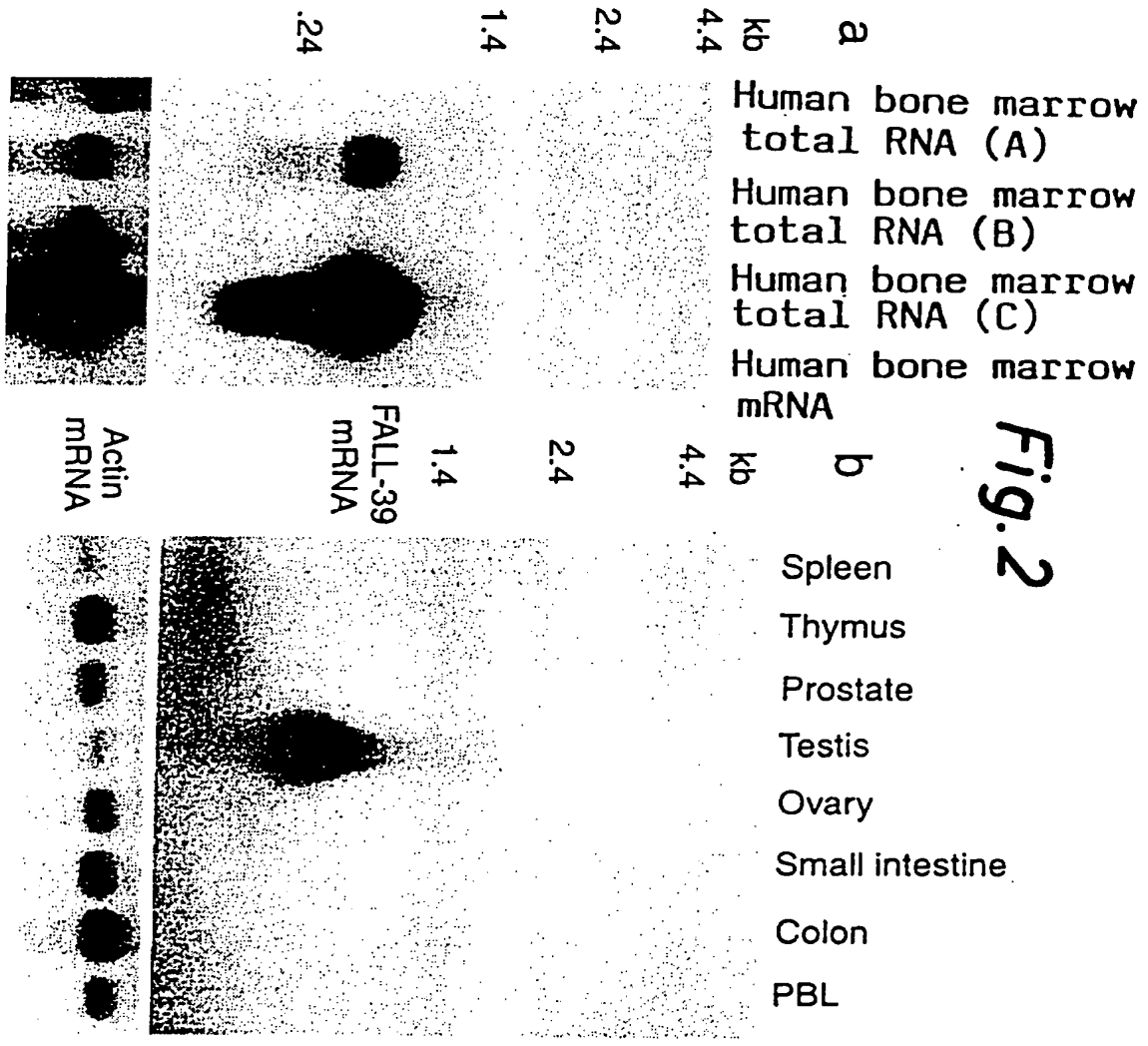
a
kb
4.4
2.4
1.4
.24
Human bone marrow total RNA (A)
Human bone marrow total RNA (B)
Human bone marrow total RNA (C)
Human bone marrow mRNA
FALL-39 mRNA
Actin mRNA
b
kb
4.4
2.4
1.4
Spleen
Thymus
Prostate
Testis
Ovary
Small intestine
Colon
PBL

Fig. 2

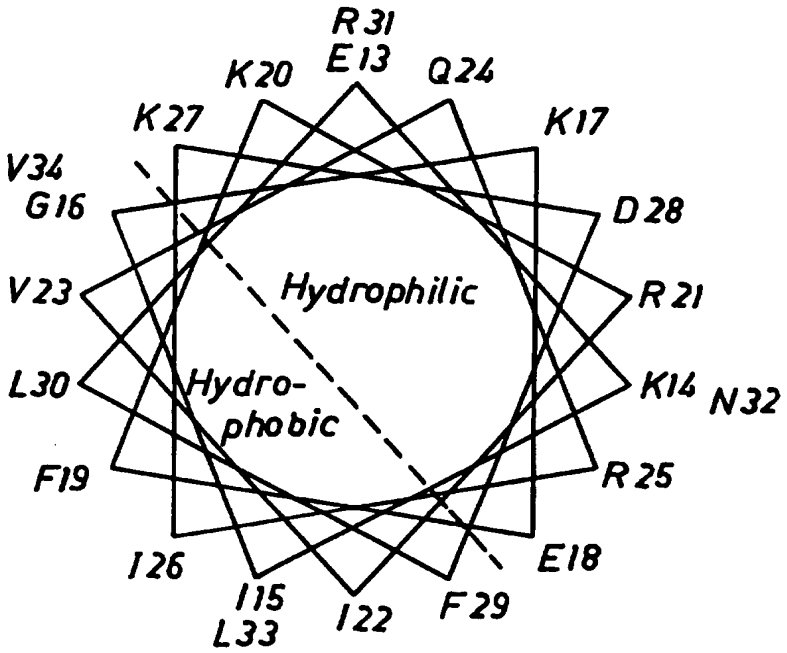
R31
E13
K20
Q24
K27
K17
V34
G16
D28
V23
Hydrophilic
R21
L30
Hydro-
phobic
K14
N32
F19
R25
I26
E18
I15
L33
I22
F29

Fig. 3

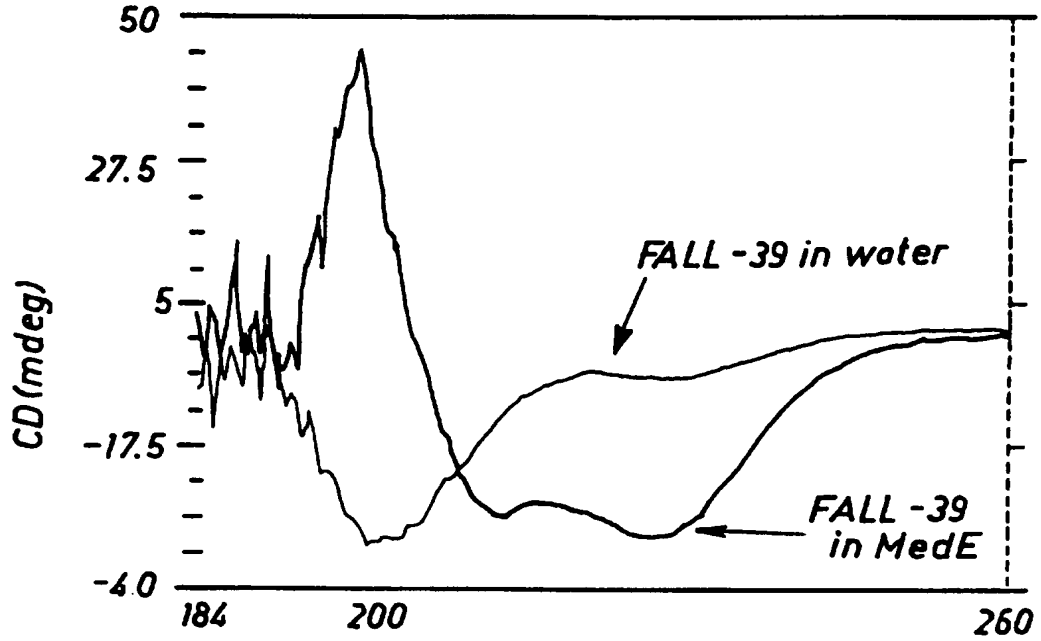
50
27.5
5
-17.5
-4.0
CD (mdeg)
FALL -39 in water
FALL -39
in MedE
184
200
260
Wave length (nm)

Fig. 4

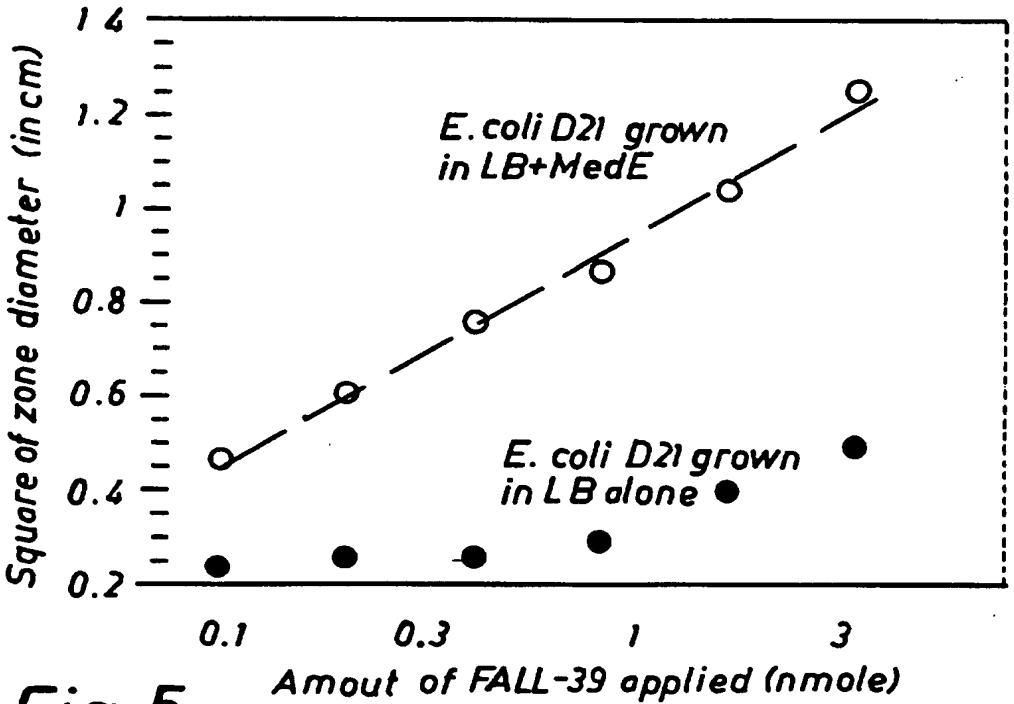
1.4
1.2
1
0.8
0.6
0.4
0.2
Square of zone diameter (in cm)
E. coli D21 grown
in LB+MedE
E. coli D21 grown
in LB alone
0.1
0.3
1
3
Amout of FALL-39 applied (nmole)

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01030

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/47, A61K 38/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE. BIOSIS. EMBASE, WPI, WPIL, US PATENT FULLTEXT DATABASES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9402589 A1 (PANORAMA RESEARCH INC.), 3 February 1994 (03.02.94), see page 9, lines 20-26; page 13, lines 10-40; claims 4 and 10	1-9,13

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 1995	22 -12- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01030

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-12
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by therapy.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01030

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9402589	03/02/94	NONE	